

# MEGAKARYOCYTE AND PLATELET DEVELOPMENT

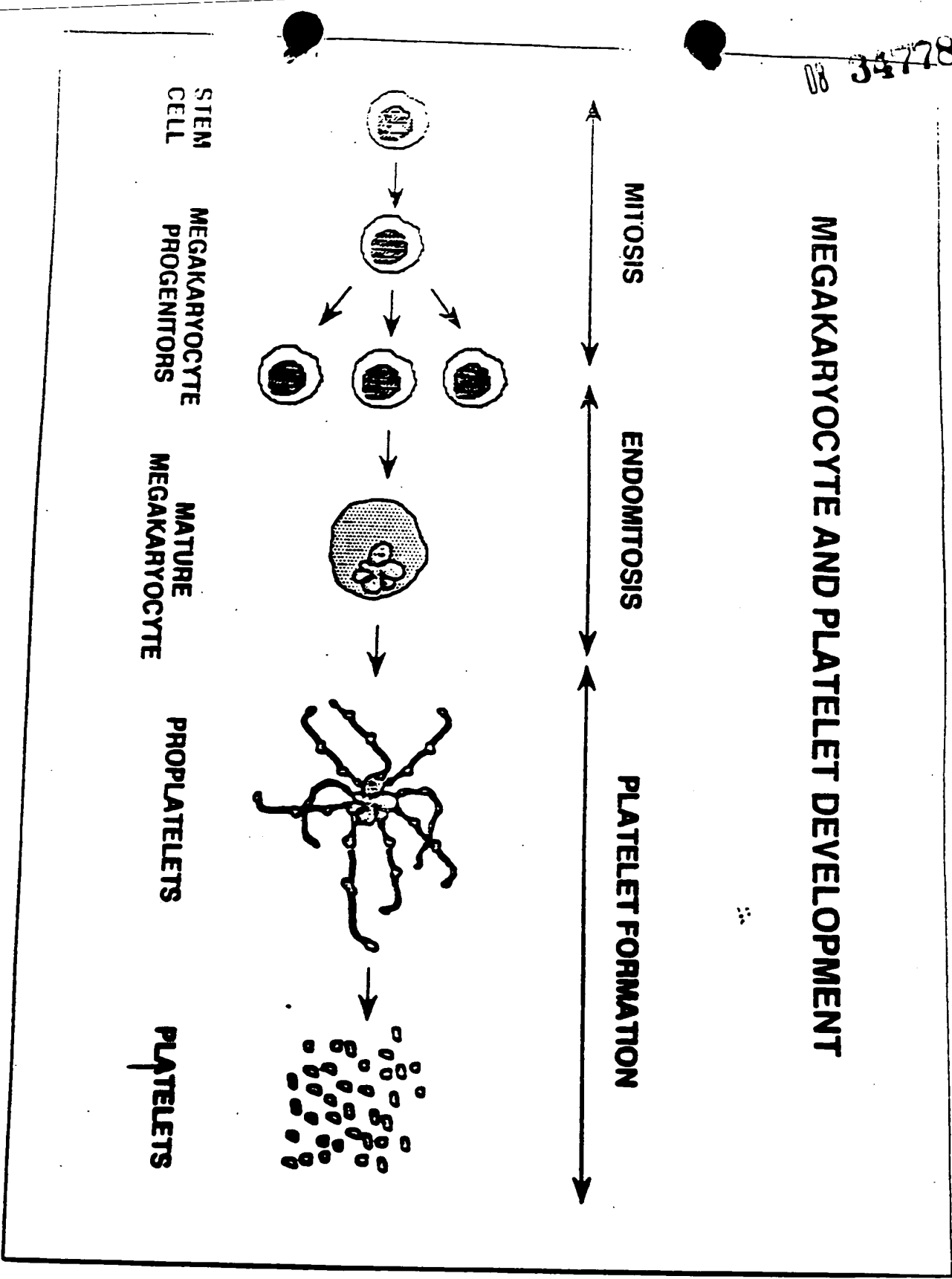


Figure 1

MPL-X completely blocks the ability of APK9 to induce megakaryocyte development

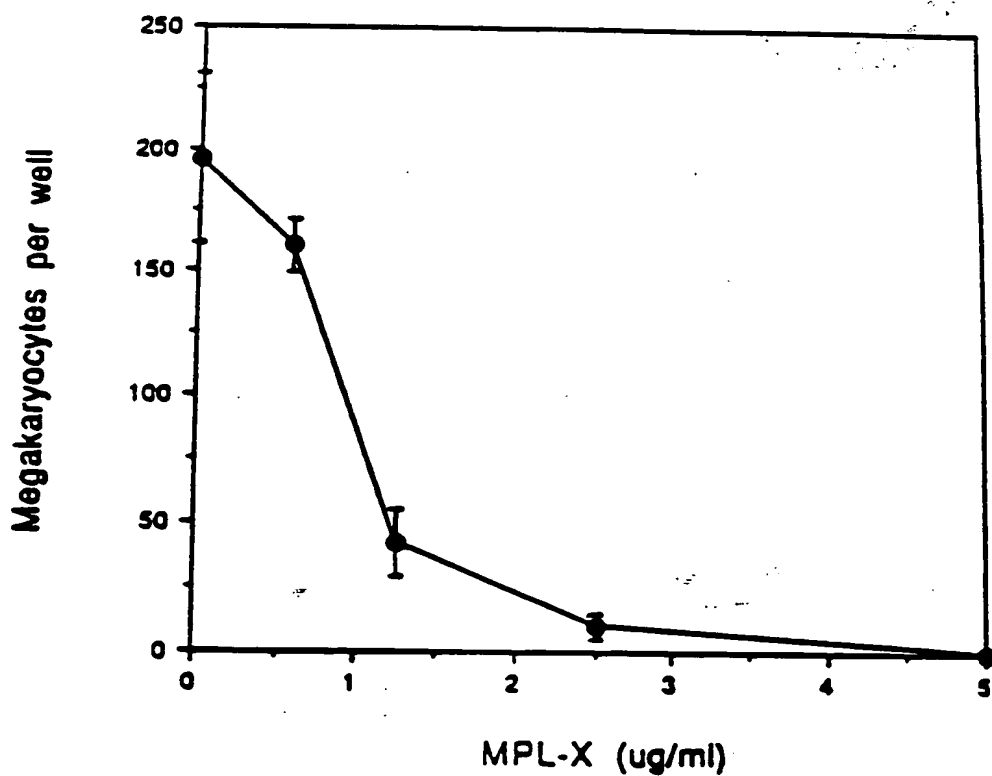


Figure 2

## MPL Ligand Stimulates 1A6.1 cell growth

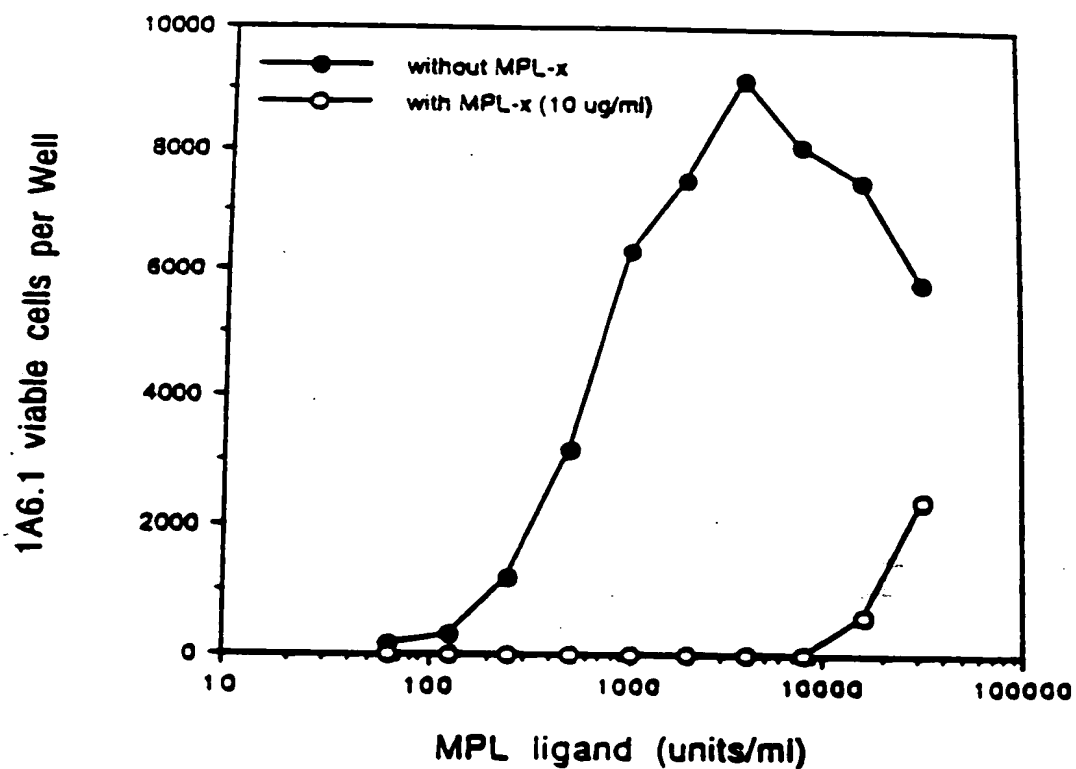


Figure 3

# Purification of Mpl ligand

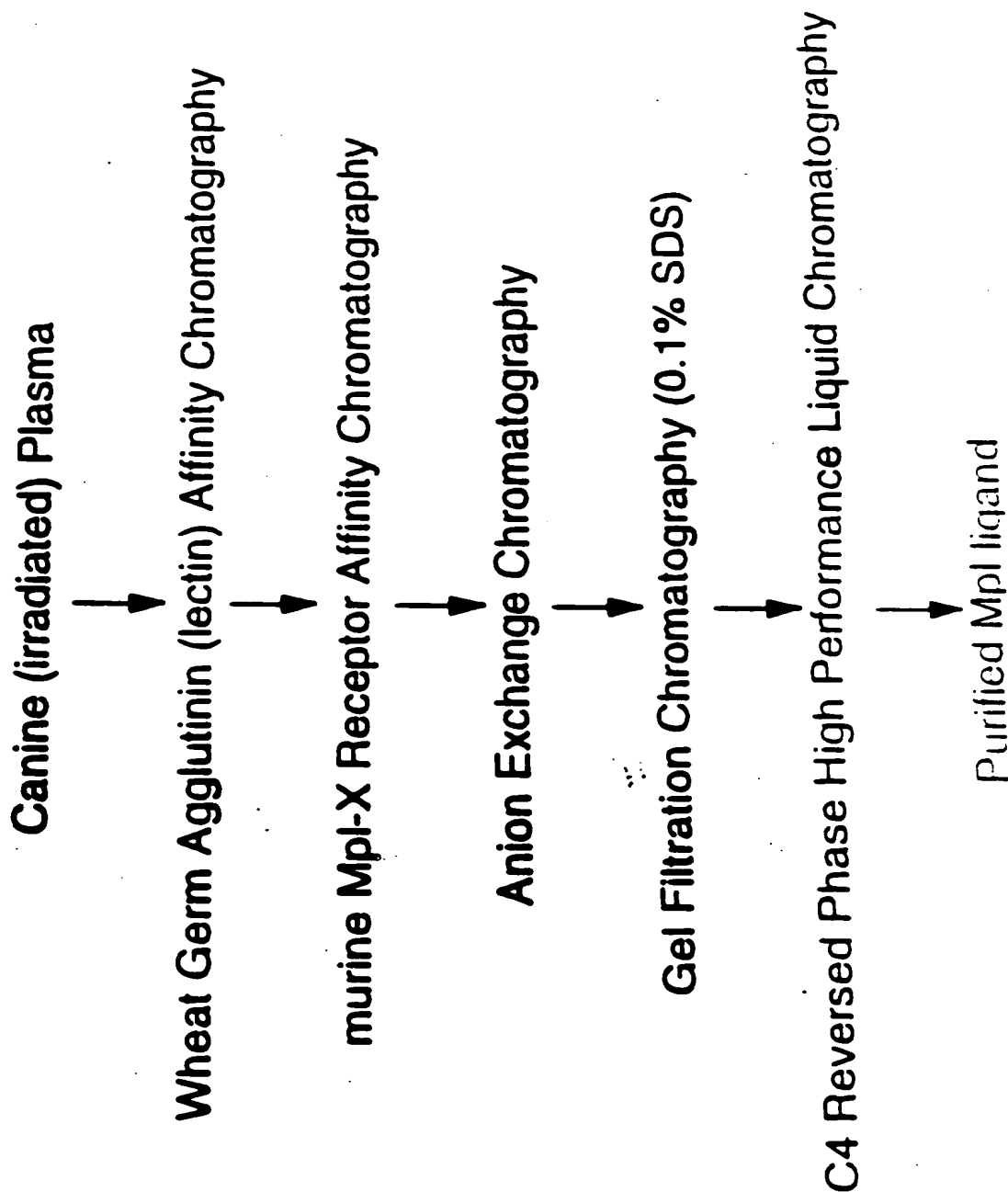
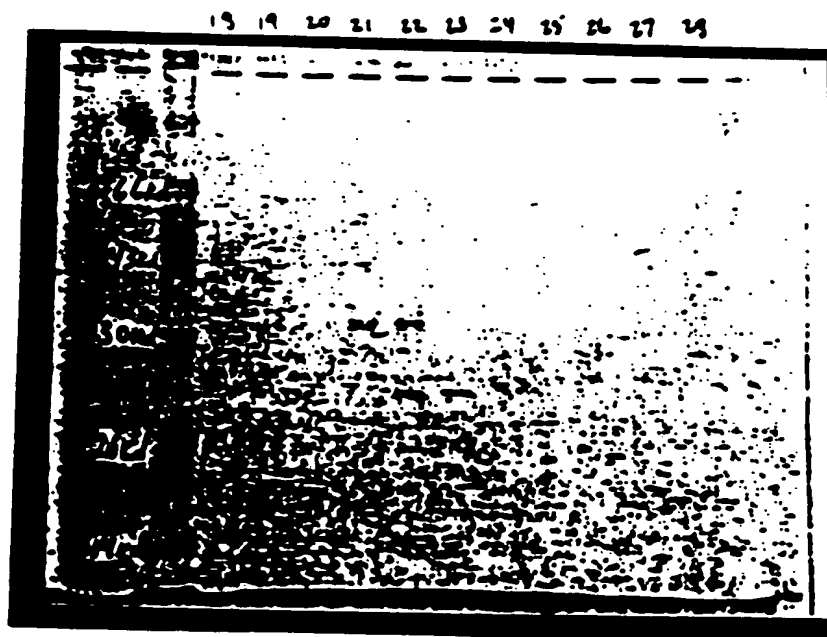
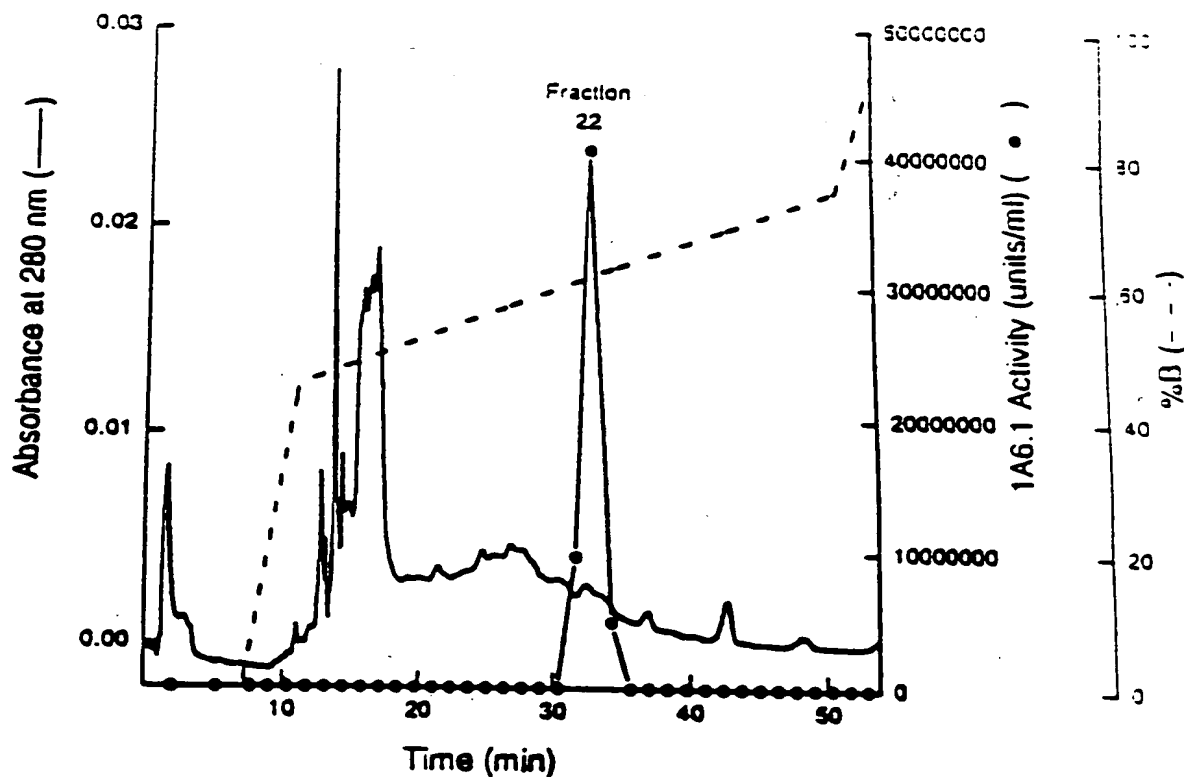


Figure 4

C4 RP-HPLC Purification of Mbl Ligand  
 Sample = Superdex 200 Fraction 42

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SDS-PAGE 14% NONREDUCING

Figure 5

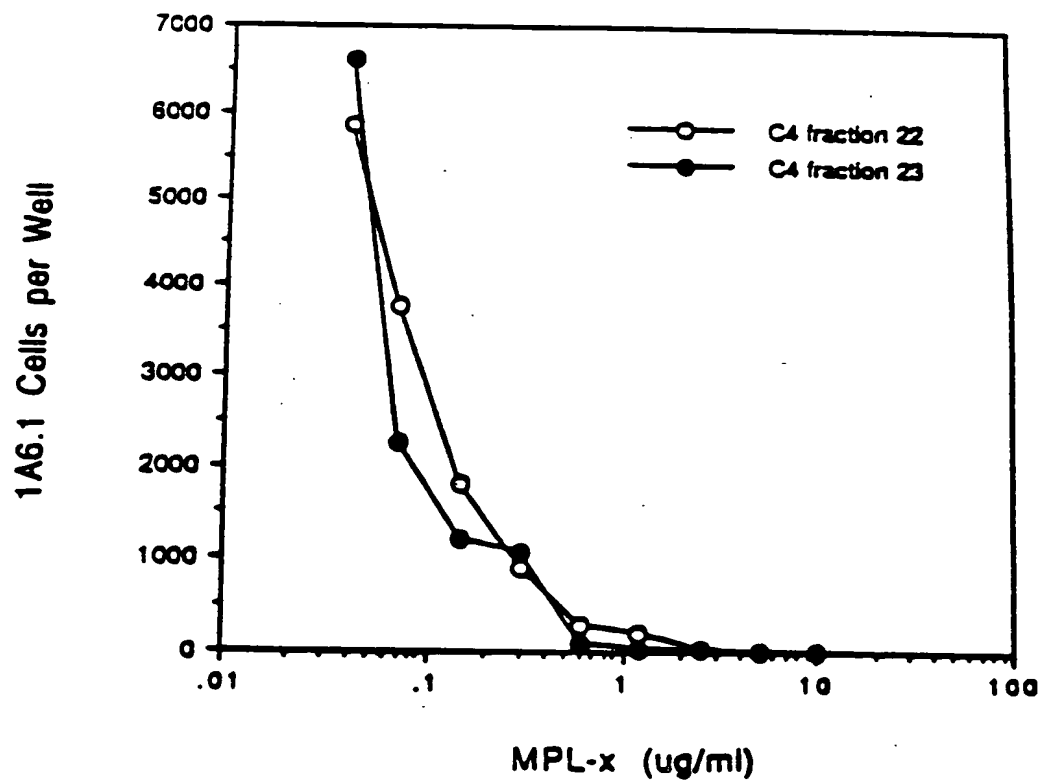


Figure 6

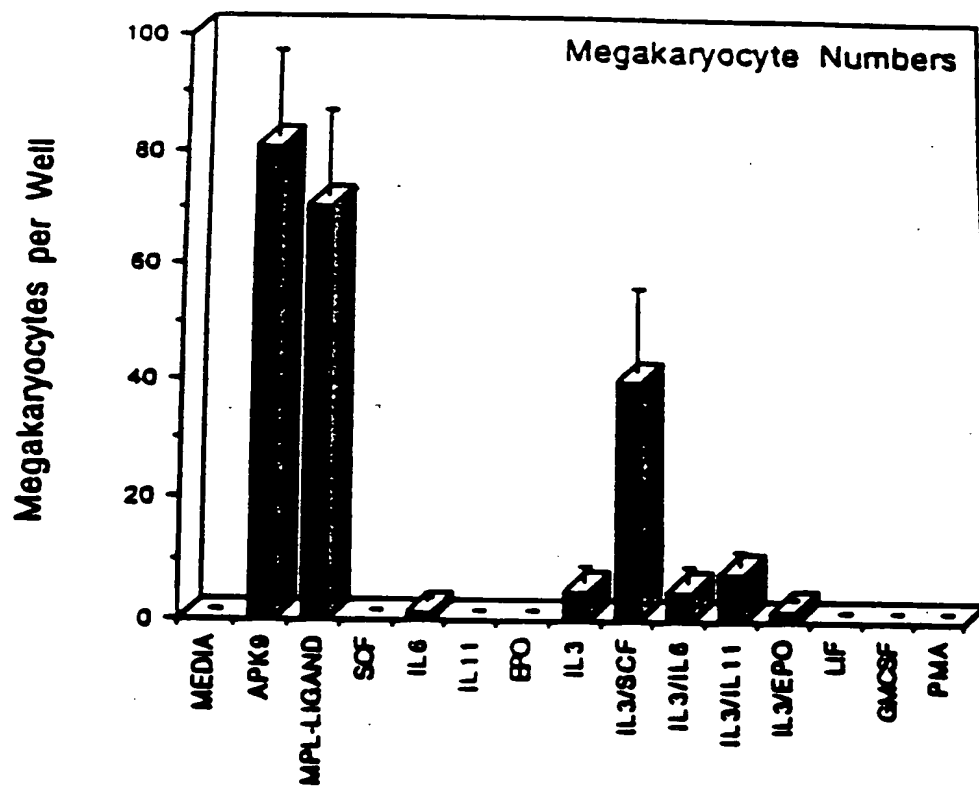


Figure 7

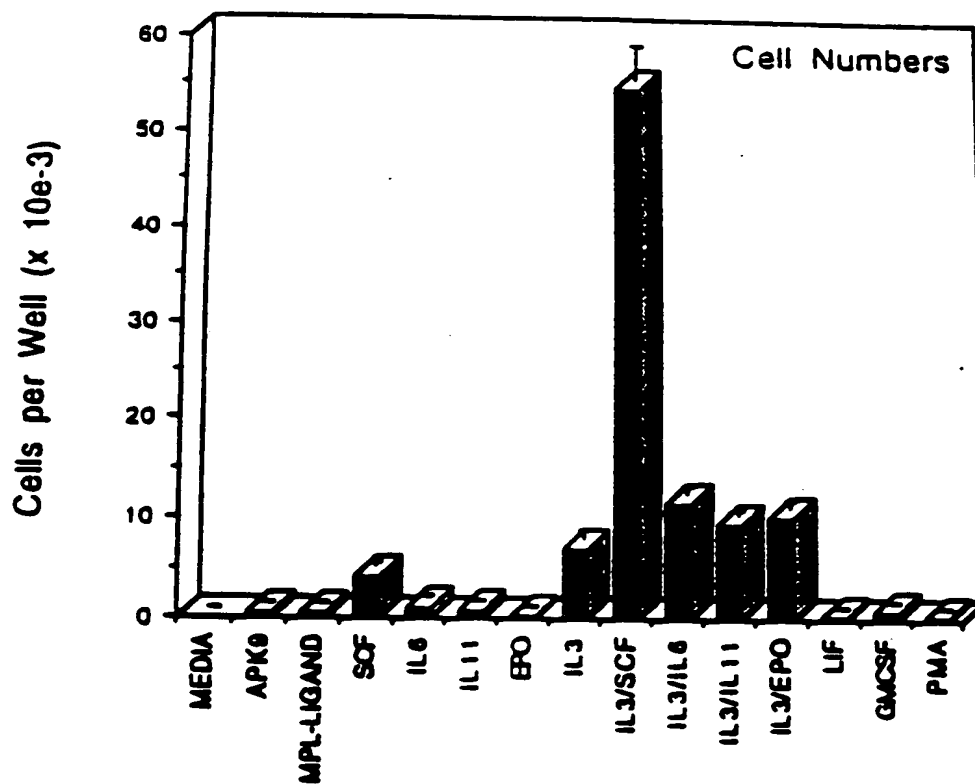


Figure 8



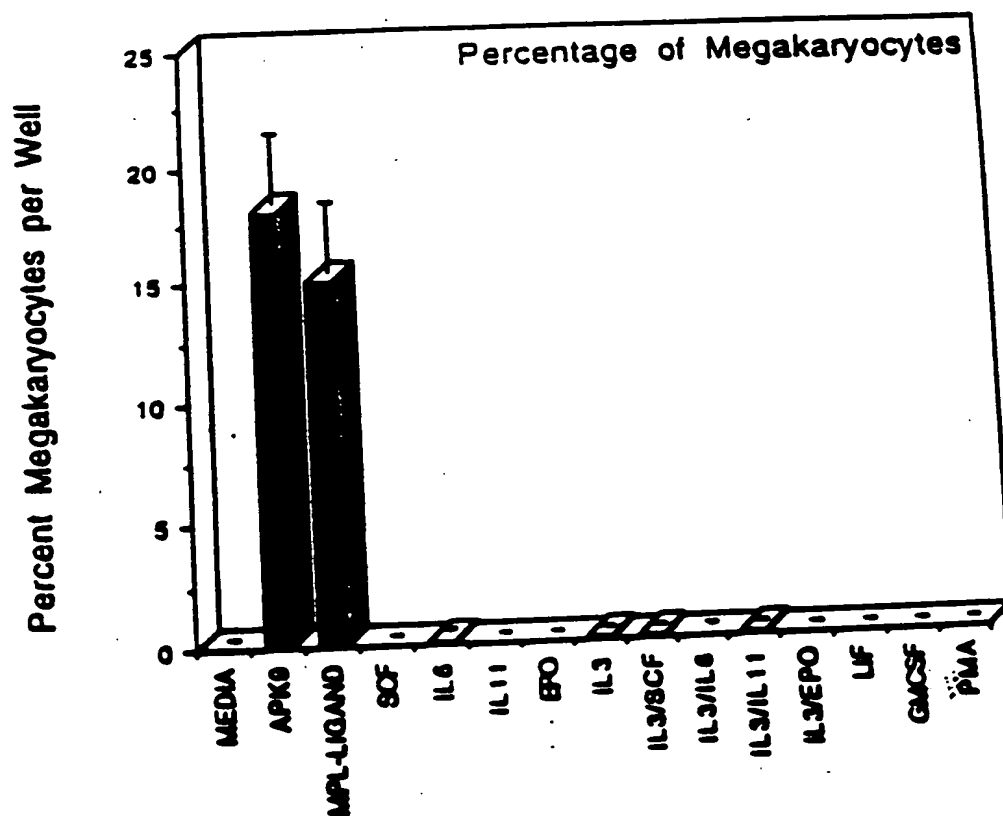


Figure 9

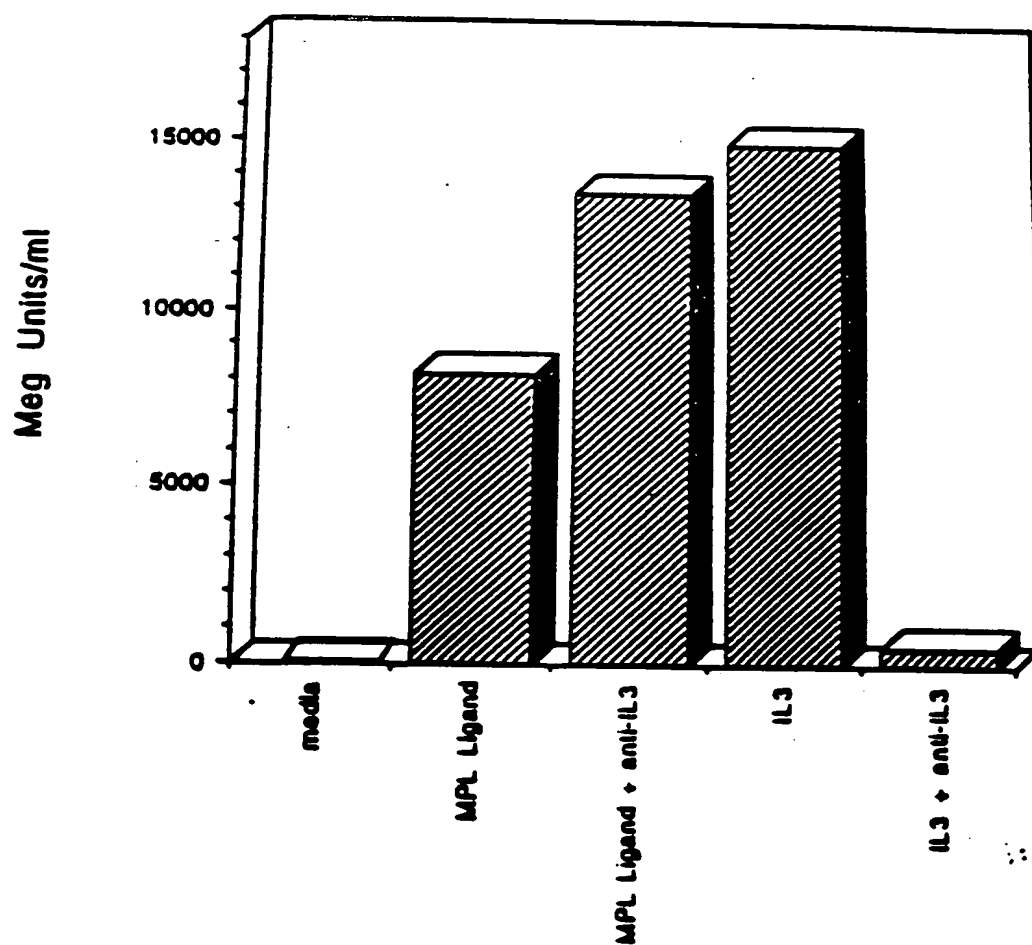


Figure 10

1	CAGGAGCCACGCGAGCGAGACACCGCGGAGATCGAGCTGACTGAATTGCTCTC	59
1	MetGlnLeuThrGlnLeuLeuLeu	8
60	GTCATCATGCTTCTCTAACTCAAGCTTAAGCTTCTAGCGCGGCTCTCTCTCTCT	119
9	ValValMetLeuLeuLeuThrAlaArgLeuThrLeuSerSer9 toAla2 toP toAlaCys	28
120	GACCTCGAGTCTCTAGTAAACTGCTTCTGACTCCCACTGCTTTCACAGCAGACTGAGC	179
29	AspLeuArgValLeuSerLysLeuLeuArgAspSerHisValLeuHisSerArgLeuSer	48
180	CAGTCCCGAGGCTTACCGTTTCCGACACCTGTCTCTCTCTCTCTCTCTCTCTCT	239
49	GlnCys9 toGluValHis9 toLeu9 toThr9 toValLeuLeu9 toAlaValAspPheSer	68
240	TTGGGAGATCGAAACCCAGTGGAGGAGACCAAGGCACAGGCATTCTGGGAGGCTG	299
69	LeuGlyGluTrpLysThrGlnMetGluGluThrLysAlaGlnAspIleLeuGlyAlaVal	88
300	ACCGTTCTCTGAGGAGTGTATGCGAGCAGCGGACAACTGGGAGCCACTTCTCTCTCA	359
89	ThrLeuLeuGluGlyValMetAlaAlaArgGlyGlnLeuGly9 toThrCysLeuSer	108
360	TCCCTCTCTGGGACAGCTTCTCTGACAGTCTCTCTCTCTCTCTCTCTCTCTCTCTCT	419
109	SerLeuLeuGlyGlnLeuSerGlyGlnValArgLeuLeuLeuGlyAlaLeuGlnSerLeu	128
420	CTTGGACCCAGCTTCTCTCAGCGGCGAGGACACAGCTCAGAGGTCCCAATGCCATC	479
129	LeuGlyThrGlnLeu9 toP toGlnGlyArgThrThrAlaHisLysAsp9 toAsnAlaIle	148
480	TTCTGAGCTTCCACACTTCTCTCGAGGAAAGGTGCGTTTCTCTGATCTTGTAGGAGG	539
149	PheLeuSerPheGlnHisLeuLeuArgGlyLysValArgPheLeuMetLeuValGlyGly	168
540	TCCACCTCTCTCTCAGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG	599
169	SerThrLeuCysValArgArgAla2 to9 toThrThrAlaValP toSerArgThrSerLeu	188
600	GTCTCAGCTGACAGCTCCCAACAGGACTTCTGATTTGTTGAGACAACTTCACT	659
189	ValLeuThrLeuAsnGluLeu9 toAsnArgThrSerGlyLeuLeuGluThrAsn9 toThr	208
660	GCTCAGCCAGACTCTGATCTCTGGCTTCTGAGTGGCAGCGGATTCAGAGCCAG	719
209	AlaSerAlaArgThrThrGlySerGlyLeuLeuLysTrpGlnGlnGlyPheArgAlaLys	228
720	ATTCTGCTCTCTGACCAACTCTCAGGTCTCTGACCAATCCCGGATCTGAC	779
229	Ile9 toGlyLeuLeuAsnGlnThrSerArgSerLeuAspGlnIle9 toGlyTyrLeuAsn	248
780	AGGATACAGGACTCTTCTGATCGACTCTCTGACTCTTCTCTGACCTCAGCGAGCT	839
249	ArgIleHisGluLeuLeuAsnGlyThrArgGlyLeuPhe9 toGly9 toSerArgArgThr	268
840	CTAGAGCGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG	899
269	LeuGlyAla9 toAspIleSerSerGlyThrSerAspThrGlySerLeu9 to9 toAsnLeu	288
900	CAGCTGAGCTCTCTCTTCCCGAACCACTCTCTCTCTCTCTCTCTCTCTCTCTCTCT	959
289	Gln9 toGlyTyrSer9 toSer9 toThrGln9 to9 toThrGlyGlnTyrThrLeuPhe9 to	308
960	CTTCCCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG	1019
309	Leu9 to9 toThrLeu9 toThr9 toValValGlnLeuHis9 toLeuLeu9 toAsp9 toSer	328
1020	GCTCCAGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG	1079
329	Ala9 toThr9 toThr9 toThr9 toSer9 toLeuLeuAsnThrSerTyrThrHisSerGlnAsn	348
1080	CTGCTCAGGAGGCTAGGTTCTCAGCACTCCCGCATCGCACTTGTCTCTCTGACG	1139
349	LeuSerGlnGluGlyAsn	353
1140	CTCCTTCTCTCAGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG	1199
1200	AAACCCAGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG	1259
1260	ACATATTAAGCTTCTGAGCTATTTTCTAGCTATCGCACTCTCTCTCTCTCTCTCT	1319
1320	AGCTCTTTCT	1342

Figure 11

## Human MDGF cDNA (no IVS 5)

1	AGGGAGCCACCCAGCCAGACACCCCGCCAGATGGAGCTGACTGAATTGCTCTCTG	60
	MetGluLeuThrGluLeuLeuVal	9
61	GTCAATGCTTCTCTAACTGCAAGGCTAACCGTGTCCAGCCCGCTCTCTCTCTGTCAC	120
10	ValMetLeuLeuLeuThrAlaArgLeuThrLeuSerSerProAlaProProAlaCysAsp	29
121	CTCCAGTCTCTAGTAACTGCTTCTGTGACTCCCATGTCTCTTACAGCAGACTGAGCCAG	180
30	LeuArgValLeuSerLysLeuLeuArgAspSerHisValLeuHisSerArgLeuSerGln	49
181	TGCCCAGAGGTTACCCCTTTGCTTACCTGTCTCTCTCTCTCTCTCTCTCTCTCTCTCT	240
50	CysProGluValHisProLeuProThrProValLeuLeuProAlaValAspPheSerLeu	69
241	GGAGATGGAAACCCAGATGGAGGAGCCAGGCCACAGGACATTCTGGAGCAGTGAC	300
70	GlyGluTrpLysThrGlnMetGluGluThrLysAlaGlnAspIleLeuGlyAlaValThr	89
301	CTTCTGCTGGAGGAGTGATGGCAGCCAGGGGACAACTGGGACCCACTTGGCTCTCATCC	360
90	LeuLeuLeuGluGlyValMetAlaAlaArgGlyGlnLeuGlyProThrCysLeuSerSer	109
361	CTCTGGGGCAGCTTCTGACAGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT	420
110	LeuLeuGlyGlnLeuSerGlyGlnValArgLeuLeuLeuGlyAlaLeuGlnSerLeuLeu	129
421	GGACCCAGCTTCTCTACAGGGCAGGACACAGCTCACAAGGATCCCATGCCATCTTC	480
130	GlyThrGlnLeuProProGlnGlyArgThrThrAlaHisLysAspProAsnAlaIlePhe	149
481	CTGAGCTTCCACACCTGCTCCGAGGAAGGACTTCTGGATTGTTGGAGCAAACTTCCAC	540
150	LeuSerPheGlnHisLeuLeuArgGlyLysAspPheTrpIleValGlyAspLysLeuHis	169
541	TGCTCAGCCAGAACTACTGGCTCTGGCTTCTGAGTGGCAGCAGGATTCCAGGCCAA	600
170	CysLeuSerGlnAsnTyrTrpLeuTrpAlaSerGluValAlaAlaGlyIleGlnSerGln	189
601	GATTCTGCTCTCTGACCAAACTCCAGGTCCCTGGACCAATCCCGGATACCTGAA	660
190	AspSerTrpSerAlaGluProAsnLeuGlnValProGlyProAsnProArgIleProGlu	209
661	CAGGATACAGAACTCTTGATGGAACTCGTGGACTCTTTCTGGACCTCAGCCAGGAC	720
210	GlnAspThrArgThrLeuGluTrpAsnSerTrpThrLeuSerTrpThrLeuThrGlnAsp	229
721	CTAGGAGCCCGGACATTCTCTAGGACATCAGACAGGCTCCCTGGCCACCACT	780
230	ProArgSerProGlyHisPheLeuArgAsnIleArgHisArgLeuProAlaThrGlnPro	249
781	CCAGCTGGAATCT	840
250	ProAlaTrpIlePheSerPheProAsnProSerSerTyrTrpThrValTyrAlaLeuPro	269
841	TCTTCCACCCAGCTTCCCAACCTCTGCTCCAGCTCCACCCCTCTCTCTCTCTCTCT	900
270	SerSerThrHisLeuAlaHisProCysGlyProAlaProProProAlaSerIle	289
901	TCTTCCACCCAGCTTCCCAACCTCTGCTCCAGCTCCACCCCTCTCTCTCTCTCTCT	960
961	TCTTCT	1020
1021	CT	1080
1081	GAACCCAAAGCTCTGAAAGGATACAGAGCTGAAAGGATATCTTTTCTCT	1140
1141	TACTTATTAACCTTCTAGCTA	1164

Figure 12

**Figure 13A**

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murine 1 MELTDLLLAAMLLAVARLTLSPPVAPACDPRLNKLRLDSSHLLHSRLSQC 50
      |||:||||.|||.|||||||:|||||:|:|||||:|||||
human 1 MELTELLLVVMLLLTARLTLSPPAPACDLRVLSKLLRDSHVLSHSRLSQC 50

51 PDVDP L S I P V L L P A V D F S L G E W K T Q T E Q S K A Q D I L G A V S I L L E G V M A A R G 100
   |:|.|||.|||.|||||||:|:|||||||:|||||||
51 P E V H P L P T P V L L P A V D F S L G E W K T Q M E E T K A Q D I L G A V T I L L E G V M A A R G 100

101 Q L E P S C L S S I L G Q L S G Q V R L L L G A L Q G L L G T Q L P L Q G R T T A H K D P M A I F L 150
   ||:|:|||||||:|||||||:|||||||:|||||||
101 Q L G P T C L S S I L G Q L S G Q V R L L L G A L Q S L L G T Q L P P Q G R T T A H K D P M A I F L 150

151 S L Q Q L L R G K V R F L L L V E G P T L C V R R T L P T T A V P S S T S Q L L T L N Q F P M R T S 200
   |:|:|||||||:|:|:|||||||:|||||||:|:|:|:|:|
151 S F Q H L L R G K V R F L M L V G G S T L C V R R A P P T T A V P S R T S L V L T L N E L P M R T S 200

201 G L L E T N F S V T A R T A G P G L L S R L Q G F R V K I T P G Q L N Q T S R S P V Q I S G Y L M R 250
   |||||...|||.|||.|||.|||.|||.|||.|||.|||.|||.|||.
201 G L L E T N T A S A R T T G S G L L K W Q Q G F R A K I . P G L L N Q T S R S L D Q I P G Y L M R 249

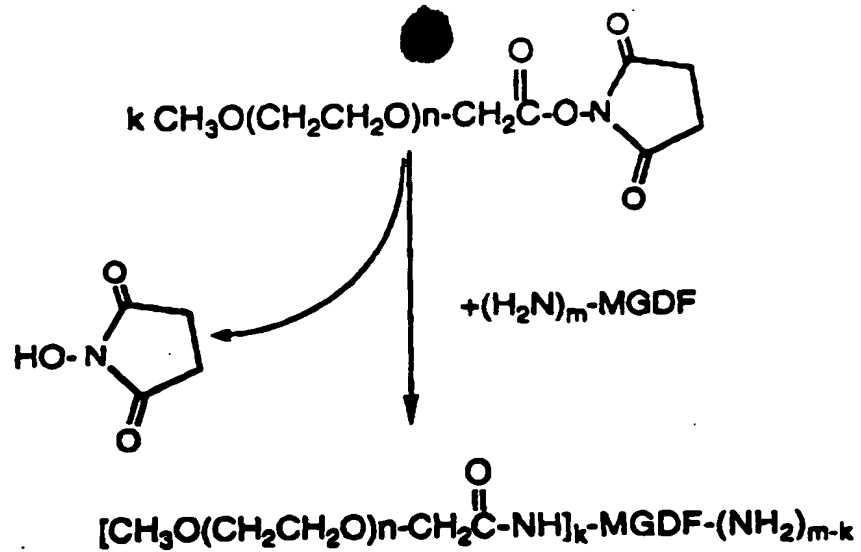
251 T H G P V N G T R G L F A G T S L Q T L E A S D I S P G A F N K G S L A F N L Q G G L P P S P S L A 300
   .|:|:||||:|:|.|||:|:|:|.|||.|||.|||.|||.|||.|||.
250 I H E L L N G T R G L F P G P S R R T L G A P D I S S G T S D T G S L P P N L Q P G Y S P S P T H P 299

301 P D G H . T P F P P S P A L P T T H G S P P Q L H P L F P D P S T T H P N S T A P H P V M K Y H P 349
   |:|:| | | |.|||.|||.|||||||:|||||.|||.|||.|||.
300 P T G Q Y T L F P L P T L P T . . . P V V Q L H P L L P D P S A P T P T P T S P L L N T S Y T H S 346

350 R N L S Q E T 356
     .|||.
347 O N L S O E G 353

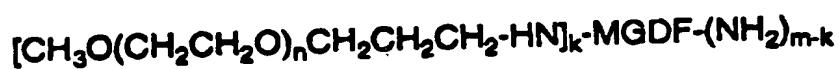
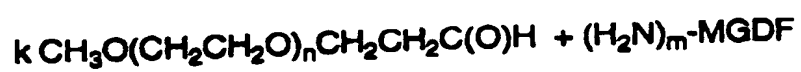
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**Figure 13B**



$k$ ,  $m$  and  $n$  are the same as defined in Figure 15.

Figure 14



- k** - number of PEG molecules reacted with a molecule of MGDF;
- n** - degree of polymerization of PEG used in the reaction; e.g.  $n=2000$  for PEG of MW=100 kD;  $n=40$  for PEG of MW=2 kD.
- m** - total number of primary amino groups per MGDF molecule.

Figure 15



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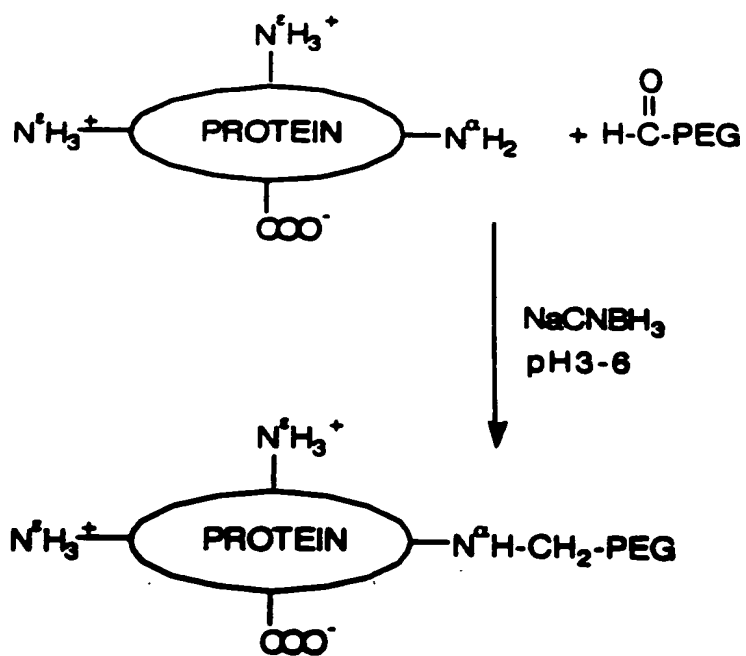


Figure 16

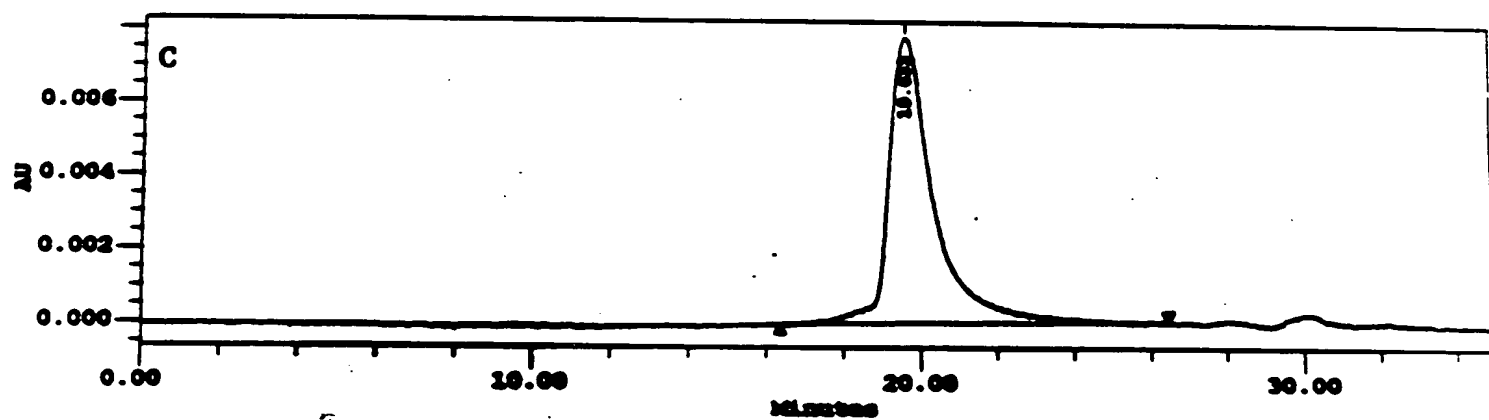
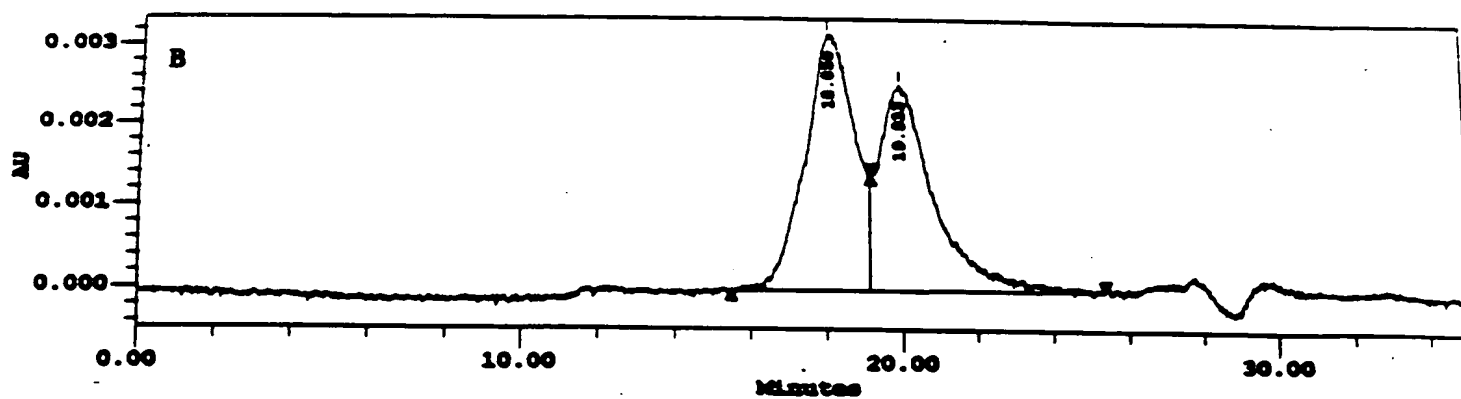
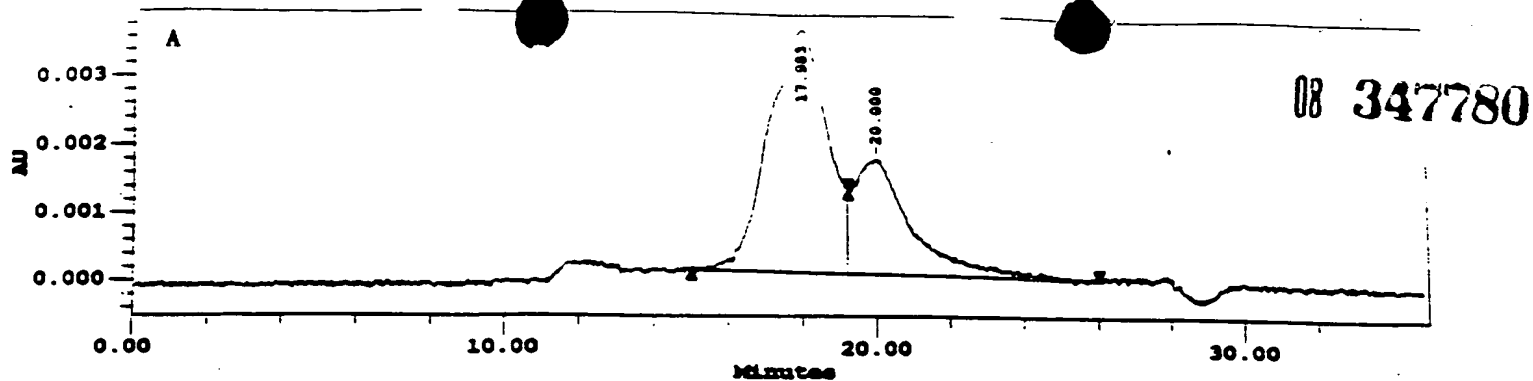


Figure 17

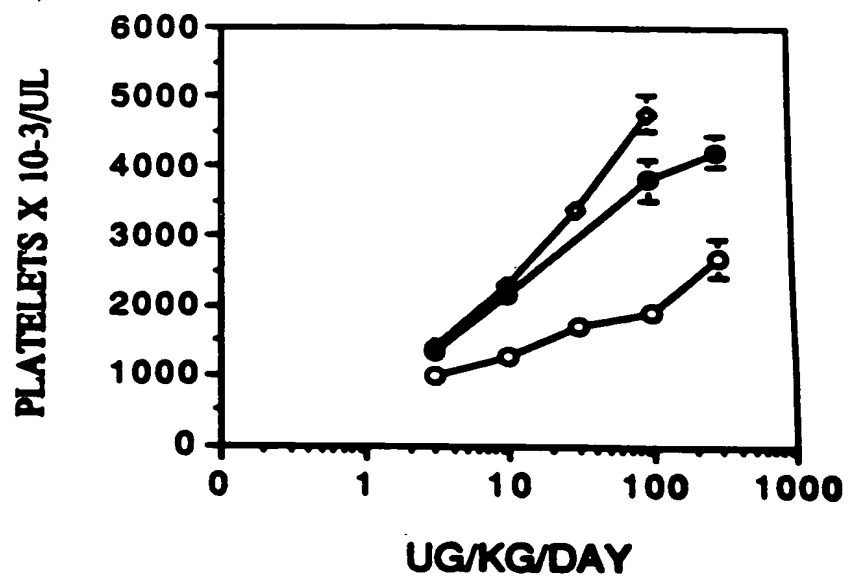


Figure 18

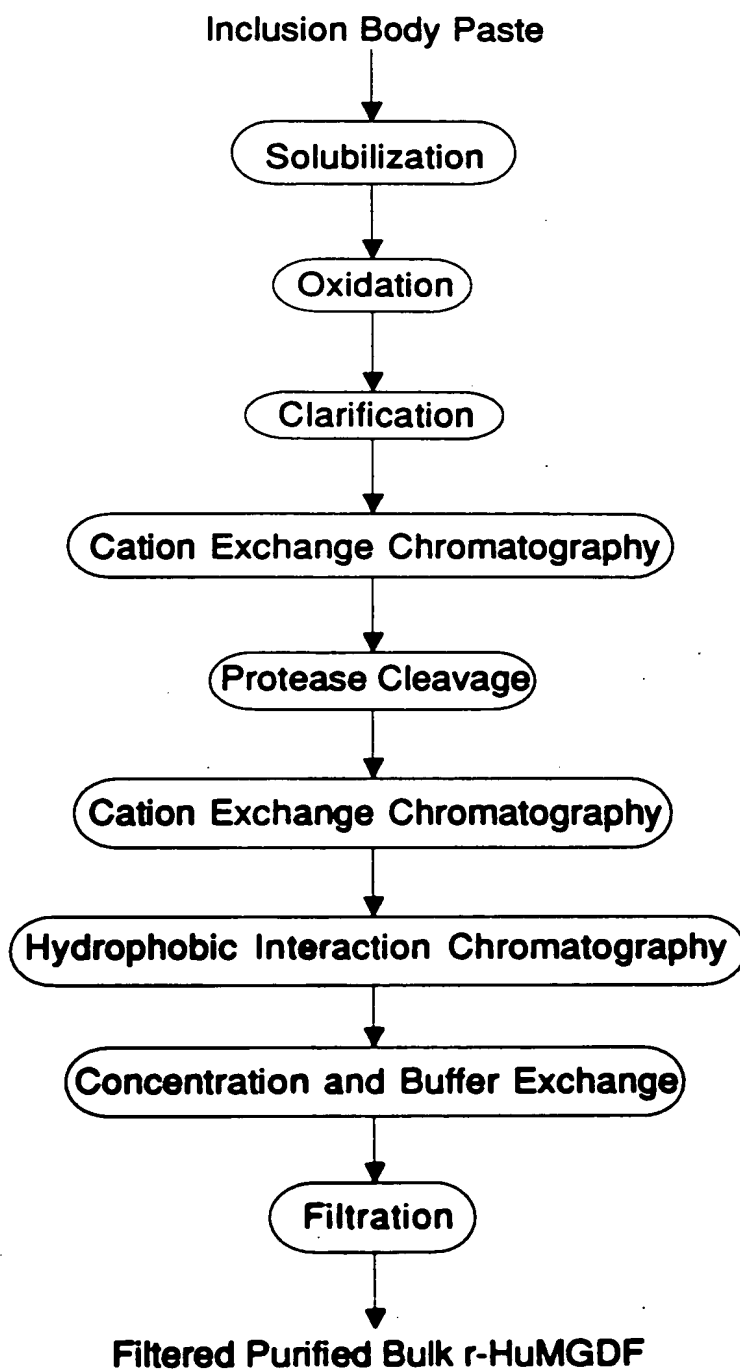
**Purification Flow Chart for r-HuMGDF**

Figure 19

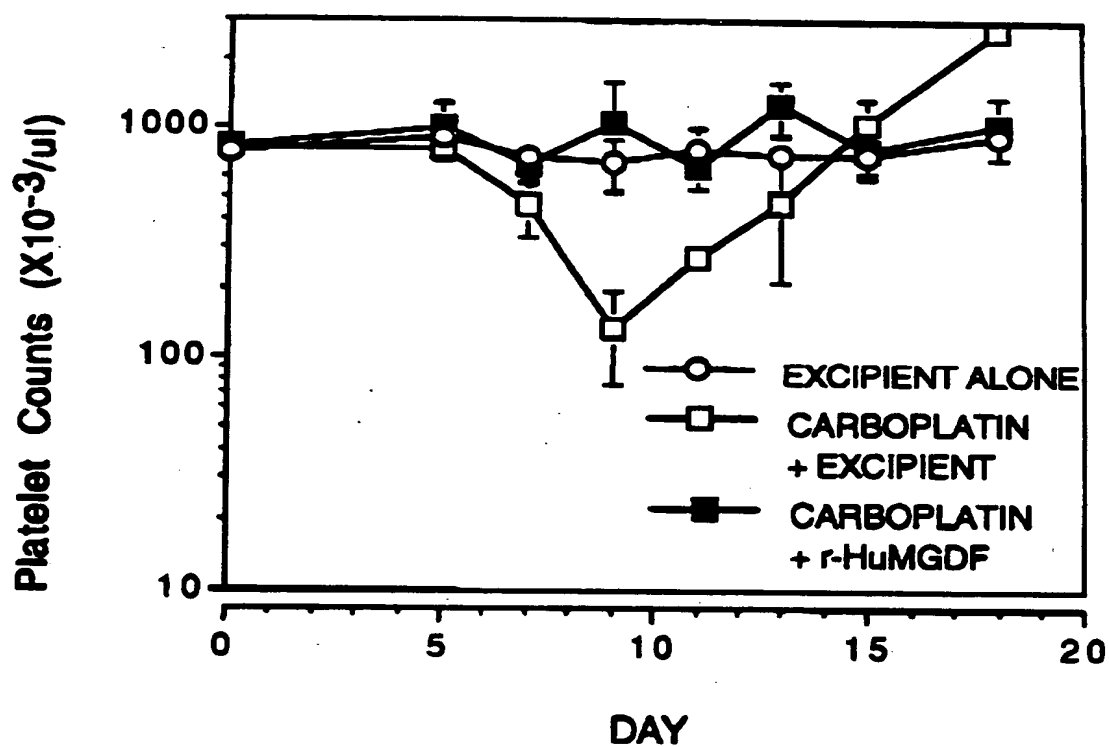


Figure 20

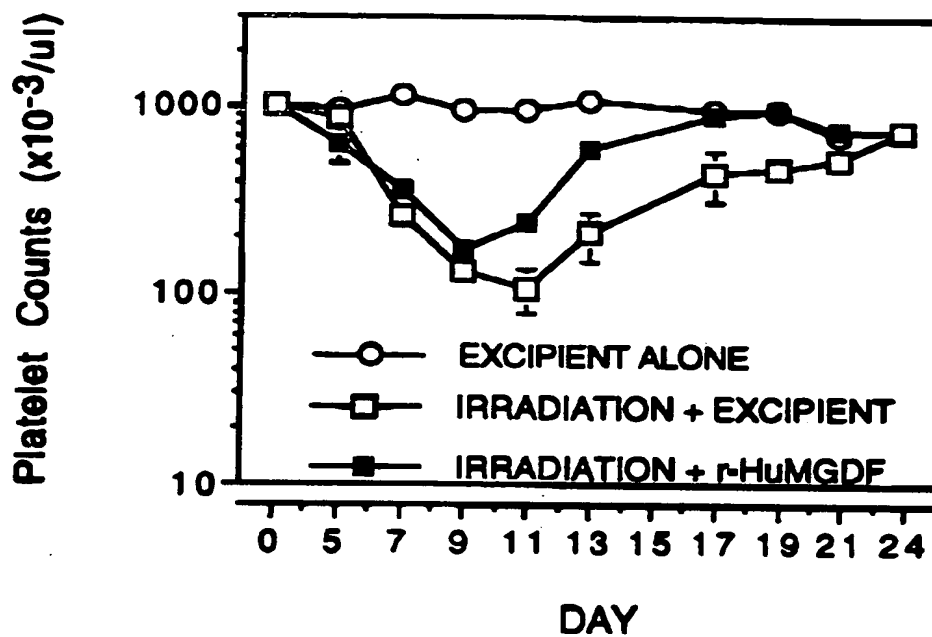


Figure 21

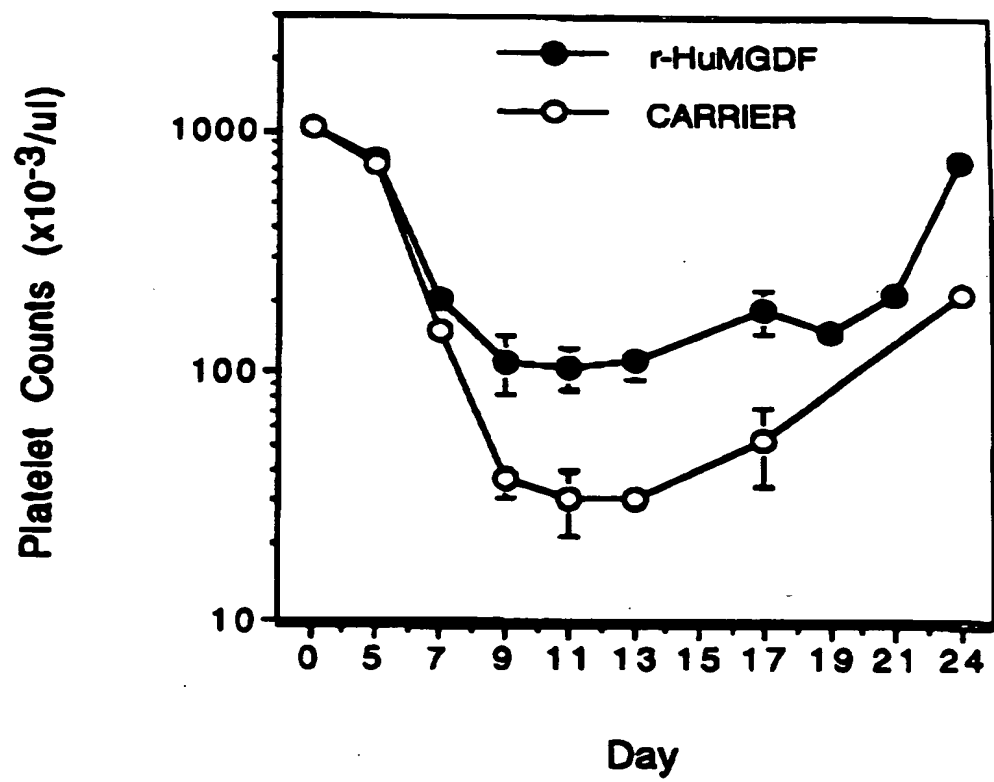


Figure 22

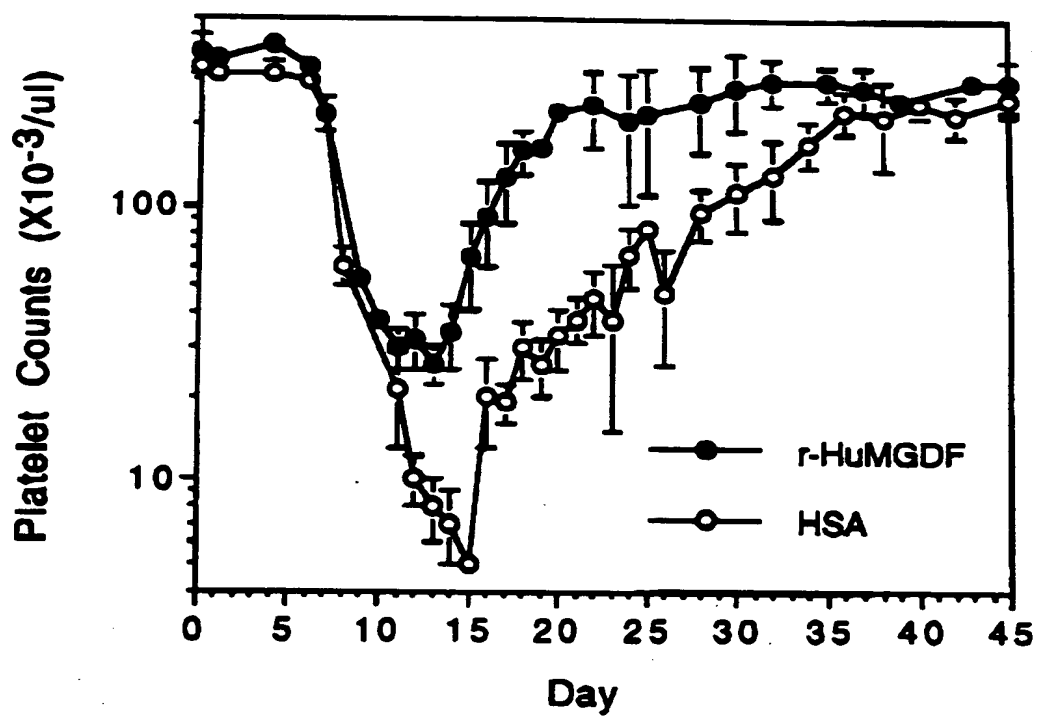


Figure 23



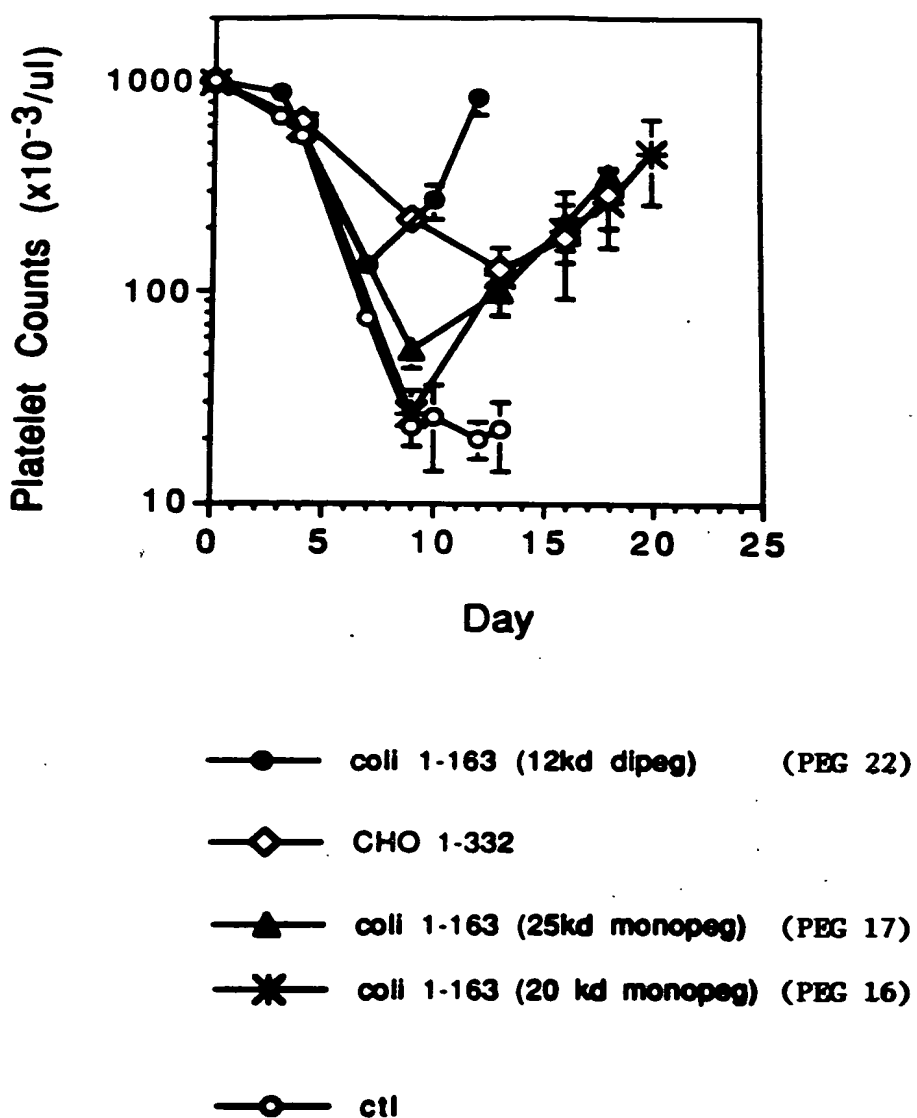


Figure 24

## r-HuMGDF (1-163) Translation

ATG	AAA	AGT	CCT	GCA	CCA	CCT	GCA	TGT	GAT	TTA	CGG	GTC	CTG
MET	LYS	SER	PRO	ALA	PRO	PRO	ALA	CYS	ASP	LEU	ARG	VAL	LEU
TCT	AAA	CTG	CTG	CGC	GAC	TCT	CAC	GTG	CTG	CAC	TCT	CGT	CTG
SER	LYS	LEU	LEU	ARG	ASP	SER	HIS	VAL	LEU	HIS	SER	ARG	LEU
TCC	CAG	TGC	CCG	GAA	GTT	CAC	CCG	CTG	CCG	ACC	CCG	GTT	CTG
SER	GLN	CYS	PRO	GLU	VAL	HIS	PRO	LEU	PRO	THR	PRO	VAL	LEU
CTT	CCG	GCT	GTC	GAC	TTC	TCC	CTG	GGT	GAA	TGG	AAA	ACC	CAG
LEU	PRO	ALA	VAL	ASP	PHE	SER	LEU	GLY	GLU	TRP	LYS	THR	GLN
ATG	GAA	GAG	ACC	AAA	GCT	CAG	GAC	ATC	CTG	GGT	GCA	GTA	ACT
MET	ALA	ALA	ARG	LYS	ALA	GLN	ASP	ILE	LEU	GLY	ALA	VAL	THR
CTG	CTT	CTG	GAA	GGC	GTT	ATG	GCT	GCA	CGT	GGC	CAG	CTT	GGC
LEU	LEU	LEU	GLU	GLY	VAL	MET	ALA	ALA	ARG	GLY	GLN	LEU	GLY
CCG	ACC	TGC	CTG	TCT	TCC	CTG	CTT	GGC	CAG	CTG	TCT	GGC	CAG
PRO	THR	CYS	LEU	SER	SER	LEU	LEU	GLY	GLN	LEU	SER	GLY	GLN
GTT	CGT	CTG	CTG	CTC	GGC	GCT	CTG	CAG	TCT	CTG	CTT	GGC	ACC
VAL	ARG	LEU	LEU	LEU	GLY	ALA	LEU	GLN	SER	LEU	LEU	GLY	THR
CAG	CTG	CCG	CCA	CAG	GGC	CGT	ACC	ACT	GCT	CAC	AAG	GAT	CCG
GLN	LEU	PRO	PRO	GLN	GLY	ARG	THR	THR	ALA	HIS	LYS	ASP	PRO
AAC	GCT	ATC	TTC	CTG	TCT	TTC	CAG	CAC	CTG	CTG	CGT	GGC	AAA
ASN	ALA	ILE	PHE	LEU	SER	PHE	GLN	HIS	LEU	LEU	ARG	GLY	LYS
GTT	CGT	TTC	CTG	ATG	CTG	GTT	GGC	GGT	TCT	ACC	CTG	TGC	GTT
VAL	ARG	PHE	LEU	MET	LEU	VAL	GLY	GLY	SER	THR	LEU	CYS	VAL
CGT	CGG	GCG	CCG	CCA	ACC	ACT	GCT	GTT	CCG	TCT	TAA		
ARG	ARG	ALA	PRO	PRO	THR	THR	ALA	VAL	PRO	SER	STOP		

Figure 25